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Signal transduction in T cells

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Rapid progress was made during the past year in the delineation of the nature of the initial biochemical events triggered by the T-cell antigen receptor. Antigen-mediated activation of phospholipase C was demonstrated to require protein tyrosine phosphorylation and, most surprising, activation of the Ras family of signal transduction molecules was shown to closely follow stimulation of the T-cell antigen receptor. Major controversy continues over which events are relevant to the various effector functions of T cells.

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Introduction

For the purposes of this review, the term T-cell activation refers to the biochemical events that lead to the initiation of interleukin (IL)-2 production after antigen becomes bound to the T-cell antigen receptor (TCR). This represents a period of 2–4 h after stimulation of T cells, which normally reside in the quiescent G0 stage of the cell cycle, and encompasses the expression of many newly transcribed genes [1•]. There are many reasons for studying mechanisms of T-cell activation, such as the rational design of novel pharmacological agents for immunosuppression and understanding some of the mechanisms of leukemogenesis. There is also potential to understand some of the mechanisms required for maintenance of tolerance and the pathological states in which tolerance is broken.

A total understanding of the molecular events of T-cell activation is far from complete. This is partly because of the complex and ill-defined structural composition of the TCR, the fact that T cells do not respond to soluble ligand but only to cell-bound ligand, and the realization that both multiple accessory surface receptors and multiple biochemical pathways appear to be activated after T-cell stimulation. This brief review will focus on the highlights of studies published since 1989. Other reviews of signal transduction in T cells that discuss earlier studies [2–4] are available.

The T-cell antigen receptor

Molecular cloning of the TCR was completed this year when the η-chain was sequenced and found to be an alternatively spliced form of the ζ-chain [5•]. Thus, the TCR consists of the heterodimeric clonotypic α- and β-chains, the CD3 γ-, δ- and ε- chains, closely related subunits encoded on chromosome 1, and the ζ- and η- chains, which are closely related and encoded on a separate chromosome.

Speculation continues about the function of the ζ- and η-chains. The chains were found to be related to the γ-chain of the hetero-trimeric Fc receptor family [6•]. Current hypotheses are that ζ- and η-chains function to couple the TCR to intracellular effector molecules such as protein tyrosine kinases.

Recently, knowledge of yet another level of complexity of the TCR has emerged. The invariant chains of the CD3 complex and ζ-dimer complex may be associated in different combinational arrays. An example of this is that the ζ-chain may consist of complexes containing either ζ–ζ, ζ–η or ζ–Fcγ dimers [7••,8••]. In addition, it appears that the TCR can assemble as subtypes containing CD3 γ- and ε-chains or CD3 δ- and ε-chains [9••–11••]. Thus, the receptor has developed a relatively well understood means of rearrangement of the clonotypic chains to achieve the diversity that is responsible for ligand binding. In addition, the TCR has developed a not yet fully appreciated

Abbreviations

APC–antigen-presenting cell, **G protein**—GTP-binding protein, **GM-CSF**—granulocyte-macrophage colony-stimulating factor, **IFN**—interferon, **IL**—interleukin, **mAb**–monoclonal antibody, **MAP-2K**—microtubule-associated protein-2-kinase, **MHC**—major histocompatibility complex, **Mls**—minor lymphocyte-stimulating determinants, **PI**—phosphoinositol, **PKC**—protein kinase C, **PLC**—phospholipase C, **PTPase**—protein tyrosine phosphatase, **TCR**—T-cell antigen receptor, **TNF**—tumor necrosis factor.

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level of structural diversity of the invariant chains that presumably allows the coupling of the TCR to different signal transduction pathways, possibly at different stages of T-cell differentiation.

Accessory receptors involved in T-cell activation

A central question concerning the role of accessory molecules in T-cell activation is whether the accessory signal enhances the signals provided by the TCR, or whether the signal is distinct from the TCR. Several studies have indicated that the function of the CD28 receptor is distinct from the TCR during the course of T-cell activation. This has been suggested partly because CD28 stimulation was shown to enhance lymphokine production even in the presence of maximal phorbol ester and calcium ionophore stimulation [12•] In addition, CD28 stimulation was shown to increase mRNA levels and secretion of IL-2, interferon (IFN)-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor (TNF)-α. These lymphokines are all induced by a combination of protein kinase C (PKC) activation provided by phorbol esters, and anti-CD28 stimulation [13•]. This means of producing IL-2 is completely resistant to suppression by cyclosporine. In contrast, the major transcriptional stimulation of IL-2 mediated by the TCR or phorbol ester plus calcium ionophore is completely abolished by cyclosporine A.

The primary function of the CD28 receptor appears to be to regulate the amount of lymphokine produced by T cells; interestingly this effect is independent of cell proliferation (DNA synthesis). Lindsten and coworkers [14••] found that the primary mechanism by which anti-CD28 augments lymphokine production in mature T cells is by inhibition of the degradation of lymphokine mRNAs. As a result of the stabilization of mRNA, the steady-state levels of lymphokines that have the T helper 1 phenotype increase, leading to enhanced translation and protein secretion. In addition to a primary effect on mRNA stability, costimulation of quiescent T cells with anti-CD3 and anti-CD28 does appear to have a number of secondary effects on T-cell responses. Late after stimulation, IL-2 mRNA levels appear to be enhanced by a CD28-dependent increase in transcription as well as mRNA stability. Studies by Weiss and colleagues [15•] have shown this to be the major mechanism by which CD28 stimulation increases lymphokine production in the Jurkat leukemia line. They identified a DNA-binding protein that binds to a site in the IL-2 promoter that is distinct from the previously described sites. Thus, it appears that CD28 may increase lymphokine production by several mechanisms. It remains to be determined whether the binding site identified by Weiss and colleagues is functional in primary T cells or whether it is a phenomenon linked to transformed cells.

One of the most important discoveries last year was the demonstration by Linsley and coworkers [16••] that a cell-surface ligand for CD28 exists on antigen-presenting cells (APCs). They have obtained convincing evidence that the ligand is the B7/BB1 molecule, an activation antigen expressed primarily on activated B7/B cells that is also a member of the immunoglobulin-gene superfamily. Thus, these results suggest that the potent effects of CD28 monoclonal antibodies (mAbs) observed *in vitro* may be mediated *in vivo* by oligomerization of the CD28 receptor by ligand presented by APC. CD28 is clearly the leading candidate to deliver a 'second signal' during T-cell activation by antigen. It is possible that this signal delivered by CD28 determines whether the cell proliferates and matures, or becomes tolerized. For example, T cells that present antigen in the context of the CD28 ligand would be expected to proliferate, whereas T cells that respond to antigen alone would not be expected to proliferate. The fact that CD28 appears during thymic ontogeny and is functional in thymocytes [17•,18•] also suggests the possibility that CD28 may be involved in positive selection the 'holy grail' of T-cell development. A recent detailed review of the CD28 receptor system is available [19]. Fig. 1 shows several potential roles of the CD28 receptor system in T-cell activation.

Signal transduction cascades

Phospholipase C

The activation of phospholipase C (PLC) has long been recognized as an important event after the binding of antigen to the TCR. The mechanism of PLC activation in T cells appears to be complex. GTP-binding proteins (G proteins) appear able to activate PLC in T cells. For example, direct activation of G proteins by aluminium fluoride causes activation of PLC in T cells [20]. In addition, Goldsmith and coworkers [21••] have found that if a known G-protein-coupled receptor is transfected into T cells, PLC activation occurs after the binding of ligand. On the other hand, there is increasing evidence that the primary means of regulating PLC activity in T cells after the binding of antigen, is by protein tyrosine phosphorylation (see below), and thus, the role of G-protein-mediated activation of PLC in T cells remains to be defined. Inokuchi and Imboden [22•] performed detailed studies of inositol phospholipid turnover, which suggest that there are multiple potential sites of regulation in the phosphoinositol (PI) cycle.

The protein tyrosine kinase—phospholipase C association

It has been clear for several years that the TCR is coupled to two important signal transduction cascades: PLC and protein tyrosine kinase activation (reviewed in [3]). A major question of T-cell activation has been whether these represent the independent activation of two pathways in parallel, or whether the TCR is primarily coupled to one of the pathways, the other pathway being activated in a serial manner. Using antiphosphotyrosine antibodies to immunoblot lysates of stimulated cells, increased

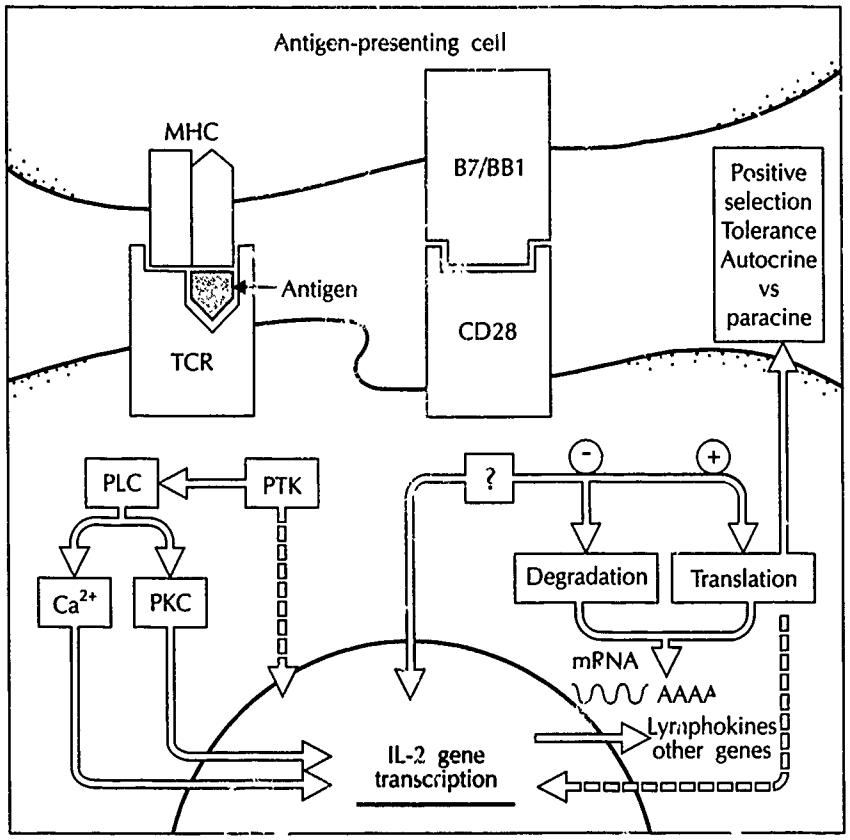

Fig. 1. A model for CD28 receptor function. The interaction of a T cell with an antigen-presenting cell engages the T-cell receptor (TCR) and the CD28 receptor. This results in signal transduction through the TCR by activation of protein tyrosine kinases (PTKs), leading to phospholipase C (PLC) activation. The signal transduction cascade activated by PLC, and possibly PTK activation, leads to initiation of IL-2-gene transcription. In the absence of the CD28 signal (−), lymphokine mRNA is labile and rapidly degraded so that little, if any, is secreted. The signal transduction pathway regulated by CD28 is unknown (?), but leads to stabilization and enhanced translation (+) of lymphokine mRNAs, so that large amounts of lymphokines are secreted. CD28 may also enhance IL-2-gene transcription, either indirectly (broken arrow) or directly (solid arrow), independent of its effects on mRNA stability. Given the cellular distribution of the CD28–B7/BB1 receptor system, this could have effects on positive selection in the thymus, tolerance induction in the thymus and peripheral T cells, and leads to an amplification of the immune response (autocrine versus paracrine) in peripheral T cells.

tyrosine phosphorylation of several substrates was detected seconds after TCR stimulation [23••]. This rapid onset of tyrosine phosphorylation preceded detection of increased PLC activity, and was unexpected as the time of TCR ζ-chain phosphorylation had been found to be relatively slow, requiring approximately 15 min from TCR stimulation. Furthermore, tyrosine phosphorylation appears to be required for PLC activation as tyrosine kinase inhibitors prevent PLC activation and subsequent events after cellular stimulation [24•,25•].

The mechanism that couples the TCR to tyrosine phosphorylation remains unclear. Samelson and coworkers [26••] have shown that the fyn kinase can be precipitated with the TCR under certain conditions. The fyn kinase is a member of the src family and is found in a variety of tissues, unlike lck which is primarily expressed in T cells. However, it was recently found that T cells express a uniquely spliced form of fyn kinase [27••]. The role of fyn in T-cell activation remains controversial for several reasons. The stoichiometry of the fyn association with the TCR appears to be low, and to date it has not been shown that stimulation of the TCR activates fyn kinase activity. In contrast, lck has high stoichiometry with the CD4 receptor, and lck kinase activity has been demonstrated to increase after CD4 crosslinking. Finally, it has not yet been shown that the fyn kinase is associated as a result the TCR in primary T cells, or whether the association is limited to T-cell lines or subtypes. It is likely that multiple tyrosine kinases are required for signal transduction through the TCR, and that both fyn and lck are involved in antigen-stimulated T-cell activation (Fig. 2)

Protein tyrosine phosphatases

The CD45 protein tyrosine phosphatase (PTPase) was shown to be required for antigen- and not IL-2 stimulated T-cell proliferation [28••]. Similarly, CD45 is required for the early biochemical events that occur after triggering of the TCR [29••]. Cell lines that lack expression of CD45 retain apparently normal expression of the TCR yet have no detectable activation of PLC or increases in calcium after TCR stimulation. Partial inhibition of tyrosine phosphatases by phenyl arsine oxide was shown to augment TCR-mediated signal transduction although higher concentrations of the drug completely inhibited signal transduction by the TCR [30•]. Thus it is likely that the CD45 PTPase is required either to activate or to maintain ac

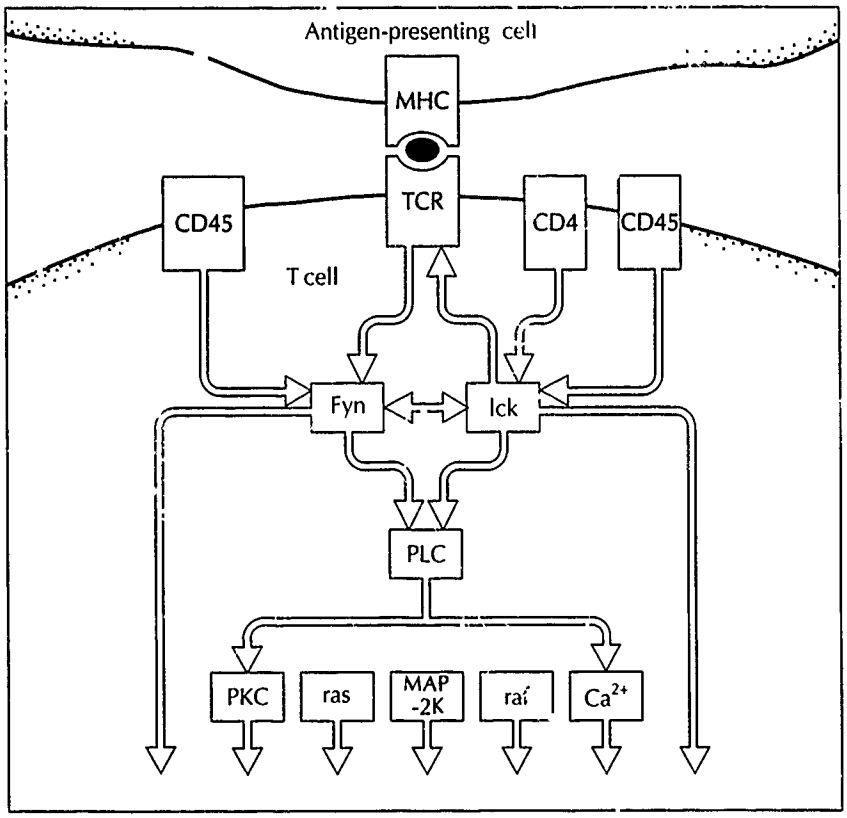

Fig. 2. The signal transduction cascade involved in antigen-specific T-cell activation. The binding of the T-cell receptor (TCR) and CD4 receptor by antigen plus major histocompatibility complex (MHC) class II antigens activates the associated protein tyrosine kinases, fyn and lck. The CD45 protein tyrosine phosphatase is required for anti-CD3-induced signal transduction. Fyn and/or lck lead to phospholipase C (PLC) activation. Protein kinase C (PKC) activation leads to ras, raf-1, and microtubule-associated protein-2 kinase (MAP-2K) activation within 5 min after TCR stimulation. It is not yet known which of these kinase signals are required for interleukin (IL)-2-gene transcription to result.

tivation of the TCR- or CD4-associated protein tyrosine kinases. Major questions remain concerning the role of the many isoforms of CD45 in various T-cell subsets. Furthermore this area is likely to become more complicated with the discovery by Cool and coworkers [31••] of a novel PTPase. This PTPase is expressed in a non-plasma membrane-bound, particulate form, and the expression of this enzyme, unlike CD45, is not restricted to leukocytes. It is reasonable to assume that each protein tyrosine kinase involved in signal transduction will have a corresponding PTPase dedicated to the regulation of the kinase activity.

Protein serine kinases

The product of the c-*raf* proto-oncogene is a serine/threonine kinase termed raf-1 that is required for growth factor-stimulated proliferation of fibroblasts [32]. Stimulation of the TCR was shown to induce raf-1 kinase activity that was maximal within 5 min of cellular stimulation [33••]. Phosphoamino acid analysis showed that TCR-induced raf-1 activation was accompanied by hyperphosphorylation on serine residues, and furthermore, was dependent upon PKC. These results were surprising because previous studies have shown that platelet derived growth factor-induced raf-1 hyperphosphorylation occurs on tyrosine residues. Thus, there appear to have evolved two distinct mechanisms of raf-1 activation, a PKC-dependent form coupled to the TCR and a tyrosine kinase-dependent form that is coupled to tyrosine growth factor receptors. It remains to be determined whether the tyrosine kinase-dependent pathway for raf-1 activation exists in T cells. An attractive possibility would be that growth factor stimulation of T cells by IL-2 or IL-4 involves raf kinase activation via a tyrosine-dependent mechanism, whereas antigen-induced activation is coupled to a distinct, serine kinase-dependent mechanism.

Nel and coworkers [34••] have recently shown that microtubule-associated protein-2-kinase (MAP-2K) is activated within minutes of TCR stimulation. In the case of MAP-2K-activation, phosphorylation on both threonine and tyrosine residues is detected. The CD4 receptor and the TCR both appear to be involved in this complex mechanism of MAP 2K activation, perhaps reflecting a dual requirement for the tyrosine kinase, lck, and a serine/threonine kinase such as PKC or raf-1.

Ras and the T cell

The ras family of G proteins are ubiquitously expressed and are required for control of cell-cycle progression. Downard and coworkers [35] demonstrated that ras activation occurs in primary T cells within minutes of TCR stimulation and that this activation is apparently dependent upon PKC activation. This is the first known example of control of ras activity by a cell surface receptor. It remains to be determined whether PKC is responsible for the ras activation. Alternative possibilities are that other

serine/threonine kinases such as raf-1 or MAP-2 kinase subserve this role.

Signal transduction and effector function

The above studies have demonstrated a role for protein tyrosine kinases in the initial events of TCR activation. What remains entirely unclear is what role, if any, tyrosine phosphorylated substrates or the second messengers of PLC activation (Inositol(1,4,5)P3 and diacylglycerol) have in the nuclear events that lead to initiation of IL-2 gene transcription. Two sides to this controversy have emerged. As mentioned above, Weiss and colleagues [21••] have transfected the Jurkat cell line with the muscarinic M1 receptor. Subsequently they found that stimulation of the muscainnic receptor activates PLC, does not lead to tyrosine kinase activation, and results in substantial secretion of IL-2 [36••]. In the same cells, stimulation of the TCR leads to tyrosine kinase activation, PLC activation and IL-2 production. These results support the conclusion that at least in some cell types, early tyrosine phosphorylation is not required for IL-2 production, and that presumably, activation of PLC and the accompanying cascades is sufficient.

Investigators at the National Institutes of Health [37] have reached a different conclusion regarding the signal transduction pathway that is involved with IL-2 gene expression. They analyzed cell mutants of the 2B4 cell line that did or did not express the ζ- and η-chains of the TCR, and found examples of cells (η-negative variants) in which activation of PLC was not detectable although normal activation of protein tyrosine kinase and normal IL-2 production was. The explanation for these differences is not immediately evident, and perhaps reflects the fact that different T-cell subtypes use distinct signal transduction pathways to initiate IL-2-gene transcription. Much experimentation will be required to determine the relative importance of these two biochemical pathways under physiological and pathological conditions. This question is of more than academic interest, as the design of specific pharmacological agents to inhibit or stimulate lymphokine production is dependent on precise knowledge of the biochemical events relevant to lymphokine production.

Conventional and 'superantigens'

It has long been known that inbred strains of mice have minor lymphocyte stimulating (Mls) genes that are potent stimulators of CD4 T-cell proliferation. The traditional assay for this effect is the stimulation of T-cell proliferation in mixed lymphocyte cultures of cells from major histocompatibility complex (MHC)-identical strains of mice. It has recently been appreciated that certain bacterial toxins can stimulate T-cell proliferation in a manner that mimics Mls antigens [38••]. T cells appear to recognize Mls and enterotoxins on entire families of the β-chain of the TCR, and thus these antigens have been termed 'super antigens' because they activate classes of T cells on the basis of the family of the β-chain expressed. T cells in the thymus that encounter endogenous Mls antigens become deleted, whereas mature T cells proliferate and produce IL-2 after Mls or enterotoxin stimulation. O'Rourke and coworkers [39] recently reported an intriguing result that suggests that the biochemical pathways used by 'conventional' antigens may differ from that of 'superantigens'. They found that stimulation of alloreactive T cells with MHC resulted in PLC activation and IL-2 production. In contrast, Mls stimulation resulted in equivalent IL-2 production and no detectable PLC activation. These findings, if confirmed, will be difficult to explain given the current models of the coupling of the TCR to signal transduction cascades. Furthermore, it is not yet known if bacterial superantigens function analogously to endogenous Mls antigens.

Conclusion

Understanding of T-cell signal transduction is still far from complete. Future work will undoubtedly uncover more receptor kinases and phosphatases that are involved in T-cell activation. There is increasing evidence to indicate that differential usage of signal transduction mechanisms may occur among T cells in different stages of differentiation. It is possible that improved understanding of signal transduction in thymocytes may yield clues to the means of positive and negative selection that determines the T-cell repertoire. The evidence reviewed herein concerning CD3 heterogeneity indicates an emerging concept of TCR domains, and the role of these domains in signal transduction, if any, remains to be appreciated.

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29. •• KORETZKY GA, PICUS J, THOMAS ML, WEISS A. **Tyrosine Phosphatase CD45 is Essential for Coupling T-Cell Antigen Receptor to the Phosphatidyl Inositol Pathway.** *Nature* 1990, 346 66–68
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30. • GARCIA-MARALES P, MINAMI Y, LUONG E, KLAUSNER RD, SAMELSON LE. **Tyrosine Phosphorylation in T Cells is Regulated by Phosphatase Activity Studies with Phenylarsine Oxide** *Proc Natl Acad Sci USA* 1990, 87:9255–9259
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31. •• COOL DE, TONKS NK, CHARBONNEAU H, WALSH KA, FISCHER EH, KREBS EG. **cDNA Isolated from a Human T-Cell Library Encodes a Member of the Protein-Tyrosine-Phosphatase Family.** *Proc Natl Acad Sci USA* 1989, 86.5257–5261
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34 •• NEL AE, POLLACK S, LANDRETH G, LEDBETTER JA, HULTIN L, WILLIAMS K, KATZ R, AKERLEY B. **CD3-Mediated Activation of MAP-2 Kinase can be Modified by Ligation of the CD4 Receptor. Evidence for Tyrosine Phosphorylation During Activation of this Kinase.** *J Immunol* 1990, 145.971–979.
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35. •• DOWNWARD J, GRAVES JD, WARNE PH, RAYTER S, CANTRELL DA: **Stimulation of p21ras upon T-Cell Activation.** *Nature* 1990, 346·719–723.
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36 •• DESAI DM, NEWTON ME, KADLECEK T, WEISS A. **Stimulation of the Phosphatidylinositol Pathway can Induce T-Cell Activation.** *Nature* 1990, 348:66–69.
This paper shows that a G-protein-coupled receptor (muscarinic type 1) when transfected into T cells can stimulate PLC and IL 2 production, in the absence of detectable protein tyrosine kinase activity.

37. • MERCEP M, BONIFACINO JS, GARCIA-MORALES P, SAMELSON LE, KLAUSNER RD, ASHWELL JD: **T Cell CD3ζ , η Heterodimer Expression and Coupling to Phosphoinoside Hydrolysis.** *Science* 1988, :571–574.
One of a series of papers by this group that demonstrates that IL 2 production can occur without detectable PLC activity. It is not yet clear how to resolve the controversy between this paper and the above study [36••]

38 •• MARRACK P, KAPPLER J. **The Staphyloccal Enterotoxins and their Relatives.** *Science* 1990, 248·705–711.
The 'superantigen' hypothesis, is summarized here by Marrack and Kappler. There is evidence from many laboratories to indicate that certain bacterial toxins and Mls antigens are recognized by the TCR in a fashion distinct from conventional antigens.

39 •• O'ROURKE AM, MESCHER MF, WEBB SR: **Activation of Polyphosphoinositide Hydrolysis in T Cells by H-2 Alloantigen but not MLS Determinants.** *Science* 1990, 249:171–174
An analysis of PI turnover, IL-2 production and T-cell proliferation after stimulation by alloantigen or Mls antigens Surprisingly, there was no detectable PI turnover after Mls stimulation, although IL 2 production was equivalent This suggests that the TCR may be able to transduce two distinct signals, and again calls into question the role of PLC activation for IL-2 production.

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